Hyperglycemia in hummingbirds and its consequences for hemoglobin glycation

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Abstract

We measured levels of glucose and glycated hemoglobin in the blood of three of the world's smallest nectarivorous birds, the Anna's (Calypte anna), Costa's (Calypte costae), and ruby-throated hummingbirds (Archilochus colubris). Plasma glucose levels of hummingbirds that were fasted overnight (17 mM) were higher than those in any mammal and are among the highest ever measured in a fasting vertebrate. Glucose levels in hummingbirds just after feeding were extreme, rising as high as 42 mM. The surprisingly high blood glucose concentrations in hummingbirds were accompanied by glycated hemoglobin levels that are the highest ever measured in birds but are lower than those of non-diabetic humans. How hummingbirds tolerate blood glucose levels that cause serious neurological and microvascular pathologies in diabetic humans and animals remains unknown. © 1998 Elsevier Science Inc. All rights reserved.

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1. Introduction

In most mammals and birds, the pancreatic hormones insulin and glucagon regulate plasma glucose concentration within a fairly narrow range, despite alternating periods of carbohydrate ingestion and fasting and variations in the rate of glucose utilization. Inadequate control of blood glucose has serious consequences. Hypoglycemia can cause loss of consciousness, convulsions, brain damage and coma [71]. Hyperglycemia, the hallmark of diabetes, results in osmotic disturbances that affect cell volume regulation and can cause dehydration via osmotic diuresis [28]. If diabetes is left untreated, plasma glucose concentration can become extremely high, even though cells are starving for glucose. The ensuing cascade of physiological and biochemical responses ultimately leads to a fall in blood pH and death from diabetic coma [28].

Less extreme but chronic disturbances in blood glucose regulation may not be immediately fatal, but they can lead to serious long-term complications that include blindness, kidney failure, nerve damage, stroke and heart disease [47,66,82]. The apparent toxicity of glucose is thought to be a consequence of damage from free radicals generated by the autoxidation of glucose, as well as by the non-enzymatic glycation of proteins, which alters their structure and causes the production of toxic ‘advanced glycation endproducts’ (AGEs) [14,40,58,60,79–81].

Because the rates of free radical production and protein glycation are proportional to glucose concentration [16], maintenance of modest plasma glucose levels would seem to be of paramount importance. Nevertheless, many species of mammals have blood glucose concentrations that are two to three times...
higher than those of non-diabetic humans, and glucose levels in birds are even higher, up to five times those of humans [61,77]. How these animals escape the devastating secondary complications that afflict diabetic humans (and other animals) with similar levels of blood glucose is unknown.

The ingestion and rapid assimilation of large amounts of sugar present a significant challenge to the physiological systems that regulate blood glucose level. The problem of blood glucose regulation should be especially acute in animals like nectarivores that consume large quantities of simple sugars. Hummingbirds support their exceptional energy requirements [74] by ingesting nectar containing sucrose, glucose and fructose [7]. They typically feed every 10–20 min throughout the day [39,72], taking nectar meals of 50–200 µl during each feeding bout [20]. Over the course of a day, a 4 g hummingbird can consume 15–20 ml of nectar containing 20–25% sucrose, ingesting an amount of sugar equal to its own body mass.

If a hummingbird is to remain in positive energy balance during the day and also accumulate the fat reserves necessary to survive the night [11,18,19,36,75], digestive speed and efficiency are essential. Nectar is half-cleared from the hummingbird’s crop in ≈ 4 min and completely cleared in < 20 min [45]. In the intestine, sucrose is hydrolyzed into glucose and fructose by the enzyme sucrase, which in hummingbirds has the highest activity ever measured in a bird [50]. Uptake of glucose from the intestine is extremely rapid; the rates of carrier-mediated glucose transport in hummingbirds are the highest among vertebrates [25,42,45]. In addition, there is mounting evidence from other nectarivorous and frugivorous birds that a significant fraction of glucose may be absorbed by carrier-independent paracellular bulk flow [1,43,44]. Assimilation efficiencies exceeding 99% are accomplished in the face of intestinal passage rates that are extremely high; non-absorbable isotopic markers ingested by hummingbirds appear in the excreta within 20 min [45]. Taken together, the remarkable physiological attributes of hummingbirds make them an ideal animal in which to explore the regulation of blood glucose levels under physiological conditions that are extreme.

In this study, we examined the regulation of blood glucose levels in three species of hummingbirds, the Anna’s (Calypte anna), Costa’s (Calypte costae), and ruby-throated hummingbirds (Archilochus colubris). We measured blood glucose levels of birds just after feeding, after daytime fasts of > 2 h, and after overnight fasts.

The fraction of plasma proteins that are glycated is commonly used as an index of the time-averaged blood glucose level over the previous weeks or months [3,5,15,38]. Because glycated hemoglobin (HbA1) is one of the most commonly used assays of protein glycation in humans and animals, we also measured levels of HbA1 in hummingbird blood.

2. Methods

We captured Anna’s hummingbirds (n = 7; mean body mass = 4.1 g) and Costa’s hummingbirds (n = 3; mean body mass = 3.5 g) in San Diego Co. and Riverside Co., California, USA; a ruby-throated hummingbird (n = 1; body mass = 3.8 g) was captured in Hancock Co., Maine, USA. The birds were maintained in flight cages on a commercial hummingbird diet (Nektar Plus; Nekton).

Blood glucose levels of fed and fasted birds were determined in separate experiments. To obtain data for ‘fed’ hummingbirds, birds with ad libitum access to food were removed from their cages and placed into jackets made from lightweight paper toweling. Most birds tolerated this procedure well, resting comfortably in the jacket and feeding readily when offered a feeder. After the bird acclimated to being fed by hand (usually 20–30 min), it was allowed to consume a meal, then a blood sample was collected immediately from a clipped toenail into a heparinized microcapillary tube. The blood was centrifuged for 5 min at 13500 × g, and the plasma was separated from the cells and stored frozen until analysis. Blood samples for fasting birds were similarly obtained from birds deprived of food overnight (10 h).

To study the time course of change in blood glucose levels during daytime fasts, it would be ideal to take multiple, sequential blood samples. Because of the hummingbird’s small body size however, it is not possible to do this without significantly compromising blood volume. Therefore, we fasted each bird for a known duration of between 0.2 and 2.3 h, and one blood sample was taken from each bird as described above. No sooner than 2 weeks later, the birds were fasted again for different durations, and another blood sample was collected. This resulted in 14 samples, 11 of which were adequate for analysis. Glucose concentration of the plasma was measured using an enzymatic, colorimetric assay (glucose oxidase, Trinder; Sigma Diagnostics Procedure No. 315). All samples were run in triplicate.

We were concerned about the possibility that handling the birds could result in stress-induced hyperglycemia. The birds were treated similarly under all protocols, and because the scatter in the data for the fed and overnight-fasted birds was small, it is unlikely that stress resulted in significant changes in glycemic status.

Whole blood for determination of glycated hemoglobin was collected from previously unbled birds into heparinized capillary tubes. The blood was
hemolyzed and the hemoglobin fractions separated by differential elution from a cation exchange resin column (Sigma Diagnostics Procedure No. 441). The glycated and non-glycated fractions were quantified spectrophotometrically, and glycated hemoglobin was expressed as a percentage of total hemoglobin.

All data are reported as the mean ± 1 S.E.

3. Results

Plasma glucose concentrations of hummingbirds just after feeding were extremely high, averaging 36 ± 2 mM in Anna’s hummingbird and 28 ± 5 mM in Costa’s; the value for the ruby-throated hummingbird was 41 mM (Fig. 1). The highest glucose level recorded in any individual was 42 mM in an Anna’s hummingbird.

Plasma glucose concentrations of Anna’s hummingbirds decreased after feeding (Fig. 2). In birds fasted for 0.25–1 h, blood glucose level varied, ranging from 19 to 29 mM. After ≈ 1.3 h of fasting, however, plasma glucose was similar among individuals and averaged 14 mM (± 0.4; Fig. 2). Glucose levels after an overnight fast were virtually identical in the three species of hummingbirds, averaging 17 mM (Fig. 1), which is slightly but not significantly more than the level after daytime fasts of > 1 h (paired t-test, P = 0.144).

Glycated hemoglobin (HbA1) levels (% total hemoglobin) were similar in Anna’s (4.6% ± 0.3) and
Costa’s (4.5% ± 0.6) hummingbirds. The value for the single ruby-throated hummingbird was 3.7% (Fig. 3a). There was no significant relationship between the fraction of glycated hemoglobin and plasma glucose concentration in either mammals or birds (least squares linear regression, \( P > 0.05 \)).

4. Discussion

4.1. Plasma glucose levels

The blood glucose concentrations of fasting hummingbirds are not extreme among birds (Fig. 3b, Fig. 4). The fasting glucose level of the great horned owl (\( Bubo virginianus \)), for example, is 21 mM, and that of the prairie falcon (\( Falco mexicanus \)) is even higher, averaging 23 mM [57]. However, the glucose levels in fasting hummingbirds are 50% greater than the highest measurements in fasting mammals (Fig. 4) and five times the normal fasting glucose concentration in humans (4.5 mM; [33]).

The glucose levels of hummingbirds immediately after feeding are extraordinary, as are the magnitudes of the excursions in glucose levels between fasting and feeding. Following a meal, the mean plasma glucose level rose by 11 and 19 mM in Costa’s and Anna’s hummingbirds, respectively, and by 24 mM in the ruby-throated hummingbird (Fig. 1). In contrast, plasma glucose concentration in chickens increases after an oral glucose load of 2 g kg\(^{-1}\), but the absolute magnitude of the change is only 5 mM (from 10 to 15 mM; [46]), and the variation in glucose levels of normally feeding birds is even less than this [10,30,76].

Blood glucose levels as high as those in hummingbirds after feeding have only been documented in other birds from individuals that were force-fed sugar solutions by intubation [48,51] or that were diabetic or experimentally pancreatized [26,55,70]. Interestingly, however, substantially higher blood glucose levels occur in amphibians subjected seasonally to extremely low temperatures. In some species of frogs, glucose in concentrations as high as 500 mM serves as a cryoprotectant during winter hibernation by promoting tissue dehydration and limiting ice formation, both of which improve survival of freezing temperatures [22,23,73].

Because of their small size and limited blood volume, it is difficult to document natural variation in blood glucose levels of freely feeding hummingbirds. However, we can expect that hummingbirds should experience the substantial changes in blood glucose observed in this study at least twice daily: at the beginning of the day when breaking the overnight fast, and at the end of the day after the last feeding bout before nightfall. During the daytime, the stability of blood glucose levels should vary with feeding frequency. In birds feeding often enough that the crop always contains nectar (i.e. every 10–20 min, based on typical meal size and crop emptying rate; [20,45]), plasma glucose levels should remain high. In birds feeding less frequently, as during migration or in individuals without a territory, glucose levels could vary considerably depending upon feeding frequency, meal size and the rates of energy expenditure and gluconeogenesis.

4.2. Hyperglycemia and glucose toxicity

Although the mechanisms underlying the toxic effects of hyperglycemia in humans and other animals remain largely unknown, research in the last decade has begun to focus on the generation of free radicals and the chemical interactions of glucose with proteins. Many proteins undergo post-translational modification by the enzymatic attachment of glucose to specific amino acid residues, and the resulting alteration of structure is critical to function. However, glucose can also condense spontaneously and non-enzymatically with proteins. These glycated proteins subsequently degrade into ketoaldehydes that can cross-link to form ‘advanced glycation endproducts’, which are toxic [15]. Not only does glycation significantly change the functional properties of the original protein, but the resulting glycated proteins and endproducts are thought to play central roles in the pathogenesis of the vascular, neural, and renal complications of diabetes [14,41,47,66,79].

Because this condensation reaction is non-enzymatic, the rate of protein glycation is strongly influenced by the ambient time-averaged glucose concentration [37]. The reaction is also essentially irreversible, so chronically elevated plasma glucose should be manifested as
Fig. 5. Allometry of red blood cell (RBC) lifespan (days) in mammals and birds. The regression for mammals is $y = 68M^{0.132}$ [78] where $M$ is body mass in kg. There are data available for only four species of birds, the chicken [13,35,65], pigeon [65], duck [65], and Japanese quail [56].

an elevated level of glycated protein. Indeed, the measurement of glycated proteins (especially hemoglobin) is now used as a means of assessing the degree of glycemic control over the previous 1–2 months in humans [60,66,82]. Poorly controlled blood glucose in humans is reflected in elevated levels of glycated hemoglobin (12–13%) relative to normal (5–7%; [68]). The degree of hemoglobin glycation has also been used to assess the body condition of migratory birds [5].

Although birds have higher levels of blood glucose than mammals, they have lower levels of glycated hemoglobin (Fig. 3a,b). In fact, although the fraction of glycated hemoglobin in hummingbird blood (2–5%) is the highest ever measured in a bird, these values would not be exceptional in a non-diabetic mammal. Glucose enters mammalian red cells by facilitated diffusion, and there is wide interspecific variation in red cell permeability to glucose [38]. In birds, on the other hand, the nucleated erythrocytes are much less permeable to glucose [67], the uptake of which is mediated by a calcium-dependent transporter [12]. Consequently, despite high extracellular glucose concentrations in birds, intracellular glucose, and therefore glycated hemoglobin, in the erythrocytes is very low [52]. In addition, the limited data available for birds suggest that the lifespan of avian erythrocytes is only 50–70% that of mammals (Fig. 5). A higher rate of red cell turnover will result in a lower steady-state fraction of glycated hemoglobin, all other things being equal [59].

The degree of non-enzymatic protein glycation can also be reduced by competitive inhibition by a number of compounds that are present in the diet or synthesized endogenously. For example, vitamin C (ascorbic acid), in addition to its role as a potent antioxidant [69], can reversibly bind to the amino groups on proteins that are the sites of glycation, thereby protecting them from interaction with glucose [24]. Most birds and mammals can synthesize vitamin C, but humans and some other primates cannot and must rely on dietary sources [21,49,54], which might account for the higher levels of glycated hemoglobin in their blood. Vitamin C is also found in the nectar of some species of plants [8], so hummingbirds might have both endogenous and dietary sources of ascorbic acid.

5. Conclusion

Hummingbirds are surprisingly long-lived—birds in the field can live as long as 12 years [17]. Yet hummingbirds tolerate, apparently without harm, levels of blood glucose that in other animals result in serious tissue damage and death. The means by which hummingbirds escape the pathologies that typically accompany hyperglycemia are unknown.

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Appendix A. Mean body mass and blood glucose concentration for bird species included in Fig. 4, arranged in order of increasing body mass

<table>
<thead>
<tr>
<th>Species</th>
<th>Body mass (kg)</th>
<th>Glucose (mM)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Costa’s hummingbird (<em>Calypte costae</em>)</td>
<td>0.003</td>
<td>17.2</td>
<td>This study</td>
</tr>
<tr>
<td>Ruby-throated hummingbird (<em>Archilochus colubris</em>)</td>
<td>0.004</td>
<td>17.8</td>
<td>This study</td>
</tr>
<tr>
<td>Anna’s hummingbird (<em>Calypte anna</em>)</td>
<td>0.005</td>
<td>16.7</td>
<td>This study</td>
</tr>
<tr>
<td>Black-capped chippadee (<em>Parus (= Sylvia) atricapillus</em>)</td>
<td>0.011</td>
<td>7.3</td>
<td>[6]</td>
</tr>
<tr>
<td>European robin (<em>Erithacus rubecula</em>)</td>
<td>0.016</td>
<td>11.2</td>
<td>[30]</td>
</tr>
<tr>
<td>Garden warbler (<em>Sylvia borin</em>)</td>
<td>0.030</td>
<td>13.1</td>
<td>[6]</td>
</tr>
</tbody>
</table>
Gray catbird (Dumetella carolinensis) 0.036 20.7 [48]
Purple-headed glossy starling (Lamprotornis purpureiceps) 0.079 16.6 [48]
European starling (Sturnus vulgaris) 0.080 19.2 [51]
Common grackle (Quiscalus quiscula) 0.100 19.2 [51]
Red-winged blackbird (Agelaius phoeniceus) 0.270 18.6 [51]
Blackheaded gull (Larus ridibundus) 0.284 14.7 [9]
Snowy egret (Egretta thula) 0.371 26.9 [38]
Rook (Corvus frugilegus) 0.450 13.1 [53]
Barn owl (Tyto alba) 0.460 13.8 [29]
Northern harrier (Circus cyaneus) 0.531 20.5 [57]
Rock pigeon (Columba livia) 0.542 9.1 [9]
Lanner falcon (Falco biarmicus) 0.570 10.7 [29]
Chicken (Gallus domesticus) 0.700 12.0 [57]
Buzzard (Buteo buteo) 0.800 20.4 [27,32]
Prairie falcon (Falco mexicanus) 0.801 23.0 [57]
White ibis (Eudocimus albus) 1.000 14.0 [38]
Herring gull (Larus argentatus) 1.100 19.7 [9,31]
Raven (Corvus corax) 1.200 19.9 [9]
Red-tailed hawk (Buteo jamaicensis) 1.224 19.3 [57]
Great horned owl (Bubo virginianus) 1.500 20.8 [55,57]
Great cormorant (Phalacrocorax carbo) 2.500 13.2 [9]
Greater flamingo (Phoenicopterus roseus) 3.000 13.2 [62]
Northern gannet (Sula bassana) 3.000 12.0 [9]
North American pelican (Pelecanus occidentalis) 3.300 9.5 [9]
Golden eagle (Aquila chrysaetos) 4.692 20.4 [57]
Chinstrap penguin (Pygoscelis antarctica) 4.800 15.6 [2,27]
Adelie penguin (Pygoscelis adeliae) 5.000 15.9 [2]
Gentoo penguin (Pygoscelis papua) 5.900 13.1 [2]
Mute swan (Cygnus olor) 10.000 6.7 [53]
Common redpoll (Carduelis (= Acanthis) flammea) 0.013 18.3 [30]
Emperor penguin (Aptenodytes forsteri) 25.300 15.6 [34]

References


